```
                                   <----------βB----------->
1        5          10         15         20         25
G  P  V  C  A  E  A  S  D  V  Y  S  P  C  M  I  A  S  T  P  P  A  P  F  S
GGACCTGTTTGTGCTGAAGCCTCAGATGTGTATAGCCCATGTATGATAGCTAGCACTCCTCCTGCTCCATTTTCA
    2670                                 2700       NheI                 2730
```

```
    <-----βC--------->
        30         35          40
D  V  T  A  V  T  F  D  L  I  N  G  K  I  T
GACGTTACAGCAGTAACTTTTGACTTAATCAACGGCAAAATAACT
                 2760
```

FIGURE 1

(A)

```
   S  T  Y  S  R  N  A  V  P  N  L  R  G  D  L  Q  V  L  A  Q  K  V  A  R  T  L  P
CTAGCACTTATAGTAGAAATGCTGTTCCTAATTTGAGAGGAGATCTTCAAGTTTTGGCTCAAAAGGTTGCTCGGACTCTTC
    GTGAATATCATCTTTACGACAAGGATTAAACTCTCCTCTAGAAGTTCAAAACCGAGTTTTCCAACGAGCCTGAGAAGGATC
                                        BgIII
```

(B)

```
1           5           10          15          <---------------------
G  P  V  C  A  E  A  S  D  V  Y  S  P  C  M  I  A  S  T  Y  S  R  N  A  V  P  N
GGACCTGTTTGTGCTGAAGCCTCAGATGTGTATAGCCCATGTATGATAGCTAGCACTTATAGTAGAAATGCTGTTCCTAAT
      2670                          2700        NheI

-------------------------------------------------->       20             25
L  R  G  D  L  Q  V  L  A  Q  K  V  A  R  T  L  P  S  T  P  P  A  P  F  S
TTGAGAGGAGATCTTCAAGTTTTGGCTCAAAAGGTTGCTCGGACTCTTCCTAGCACTCCTCCTGCTCCATTTTCA
        BgIII                                   xNheI                2730
```

FIGURE 2

```
   <---------βB--------->
11                    136
 Y  S  P  C  M  I  A  S  T  Y  S  R  N  A  V  P  N  L  R  G  D  L  Q  V  L  A
TATAGCCCATGTATGATAGCTAGCACTTATAGTAGAAATGCTGTTCCTAATTTGAGAGGAGATCTTCAAGTTTTGGCT
ATATCGGGTACATACTATCGATCGTGAATATCATCTTTACGACAAGGATTAAACTCTCCTCTAGAAGTTCAAAACCGA
                   Nhel                                         Bgl11
```

```
                                                            <-------βC-------->
                   160         20                                   30
 Q  K  V  A  R  T  L  P  S  T  P  P  A  P  F  S  D  V  T  A  V  T  F  D  L  I
CAAAAGGTTGCTCGGACTCTTCCTAGCACTCCTCCTGCTCCATTTTCAGACGTTACAGCAGTAACTTTTGACTTAATC
GTTTTCCAACGAGCCTGAGAAGGATCGTGAGGAGGACGAGGTAAAAGTCTGCAATGTCGTCATTGAAAACTGAATTAG
                   xNhel
```

FIGURE 3

```
  15                  20                  25                  30                  35
P  C  M  I  A  S  T  P  P  A  P  F  S  D  V  T  A  V  T  F  D  L  I
CCATGTATGATAGCTAGCACTCCTCCTGCTCCATTTTCAGACGTTACAGCAGTAACTTTTGACTTAATC
2700        NheI                    2730            *                   2760
```

Site-directed Mutagenesis

```
  15                  20                  25                  30                  35
P  C  M  I  A  S  T  P  P  A  P  F  S  D  V  T  A  V  T  F  D  L  I
CCATGTATGATAGCTAGCACTCCTCCTGCTCCATTTTCAGACGTCACAGCAGTAACTTTTGACTTAATC
2700        NheI                    2730    AatII                       2760
```

FIGURE 4

(A)

```
  S   T   D   R   P   E   G   I   E   E   E   G   G   E   R   D   R   D   R   S   D
CTAGCACTGACCGCCCTGAGGGCATCGAGGAAGAGGGCGGTGAGCGCGATCGTGATCGTTCGGACGT
    GTGACTGGCGGGACTCCCGTAGCTCCTTCTCCCGCCACTCGCGCTAGCACTAGCAAGCC
                                              Pvul
```

(B)

```
1           5           10          15              <------------------
G   P   V   C   A   E   A   S   D   V   Y   S   P   C   M   I   A   S   T   D   R   P   E   G   I   E
GGACCTGTTTGTGCTGAAGCCTCAGATGTGTATAGCCCATGTATGATAGCTAGCACTGACCGCCCTGAGGGCATCGAG
     2670                                 2700          Nhel
```

```
------------------------------->                  30            35
E   E   G   G   E   R   D   R   D   R   S   D   V   T   A   V   T   F   D   L   I
GAAGAGGGCGGTGAGCGCGATCGTGATCGTTCGGACGTCACAGCAGTAACTTTTGACTTAATC
                 Pvul           AatII                      2760
```

FIGURE 5

(A)

```
  S  T  P  A  T  G  I  D  N  H  R  E  A  K  L  D
CTAGCACTCCTGCTACTGGAATCGATAATCATAGAGAAGCTAAATTGGACGT
    GTGAGGACGATGACCTTAGCTATTAGTATCTCTTCGATTTAACC
                   ClaI
```

(B)

```
1           5             10             15              <------------------
G  P  V  C  A  E  A  S  D  V  Y  S  P  C  M  I  A  S  T  P  A  T  G  I  D  N
GGACCTGTTTGTGCTGAAGCCTCAGATGTGTATAGCCCATGTATGATAGCTAGCACTCCTGCTACTGGAATCGATAAT
      2670                            2700        NheI                    ClaI
```

```
------------------>                30             35
 H  R  E  A  K  L  D  V  T  A  V  T  F  D  L  I
CATAGAGAAGCTAAATTGGACGTCACAGCAGTAACTTTTGACTTAATC
                  AatII                  2760
```

FIGURE 6

```
    <---------βB--------->
11                      140
 Y  S  P  C  M  I  A  S  T  V  P  N  L  R  G  D  L  Q  V  L  A
TATAGCCCATGTATGATAGCTAGCACTGTTCCTAATTTGAGAGGAGATCTTCAAGTTTTGGCT
ATATCGGGTACATACTATCGATCGTGACAAGGATTAAACTCTCCTCTAGAAGTTCAAAACCGA
                   NheI                      BglII
```

```
                               <------βC--------->
                 160              30
 Q  K  V  A  R  T  L  P  D  V  T  A  V  T  F  D  L  I
CAAAAGGTTGCTCGGACTCTTCCTGACGTCACAGCAGTAACTTTTGACTTAATC
GTTTTCCAACGAGCCTGAGAAGGACTGCAGTGTCGTCATTGAAAACTGAATTAG
                           AatII
```

FIGURE 7

```
   20                                                                         25
 S  T  P  P  A                                                             P  P  S  D
CTAGCACTCCTCCTGCT                                                        CCATTTTCAGACGT
    GTGAGGAGGACGA                                                        GGTAAAAGTC
```

```
141
 V   P   N   L   R   G   D   L   Q   V   L   A   Q   K   V   A   R   T   L
 GTTCCTAATTTGAGAGGAGATCTTCAAGTTTTGGCTCAAAAGGTTGCTCGGACTCTT
 CAAGGATTAAACTCTCCTCTAGAAGTTCAAAACCGAGTTTTCCAACGAGCCTGAGAA
```

FMDV-V

```
85                                        98
 K   D   A   T   G   I   D   N   H   R   E   A   K   L
AAAGATGCTACTGGAATCGATAATCATAGAGAAGCAAAATTG
TTTCTACGATGACCTTAGCTATTAGTATCTCTTCGTTTTAAC
```

HRV-II

```
731                                                                 752
 P   R   G   P   D   R   P   E   G   I   E   E   E   G   G   E   R   D   R   D   R   S
 CCTAGAGGACCAGACAGACCTGAAGGAATAGAAGAGGAAGGTGGAGAACGCGATCGAGATAGATCA
GGATCTCCTGGTCTGTCTGGACTTCCTTATCTTCTCCTTCCACCTCTTGCGCTAGCTCTATCTAGT
```

HIV-III

FIGURE 8

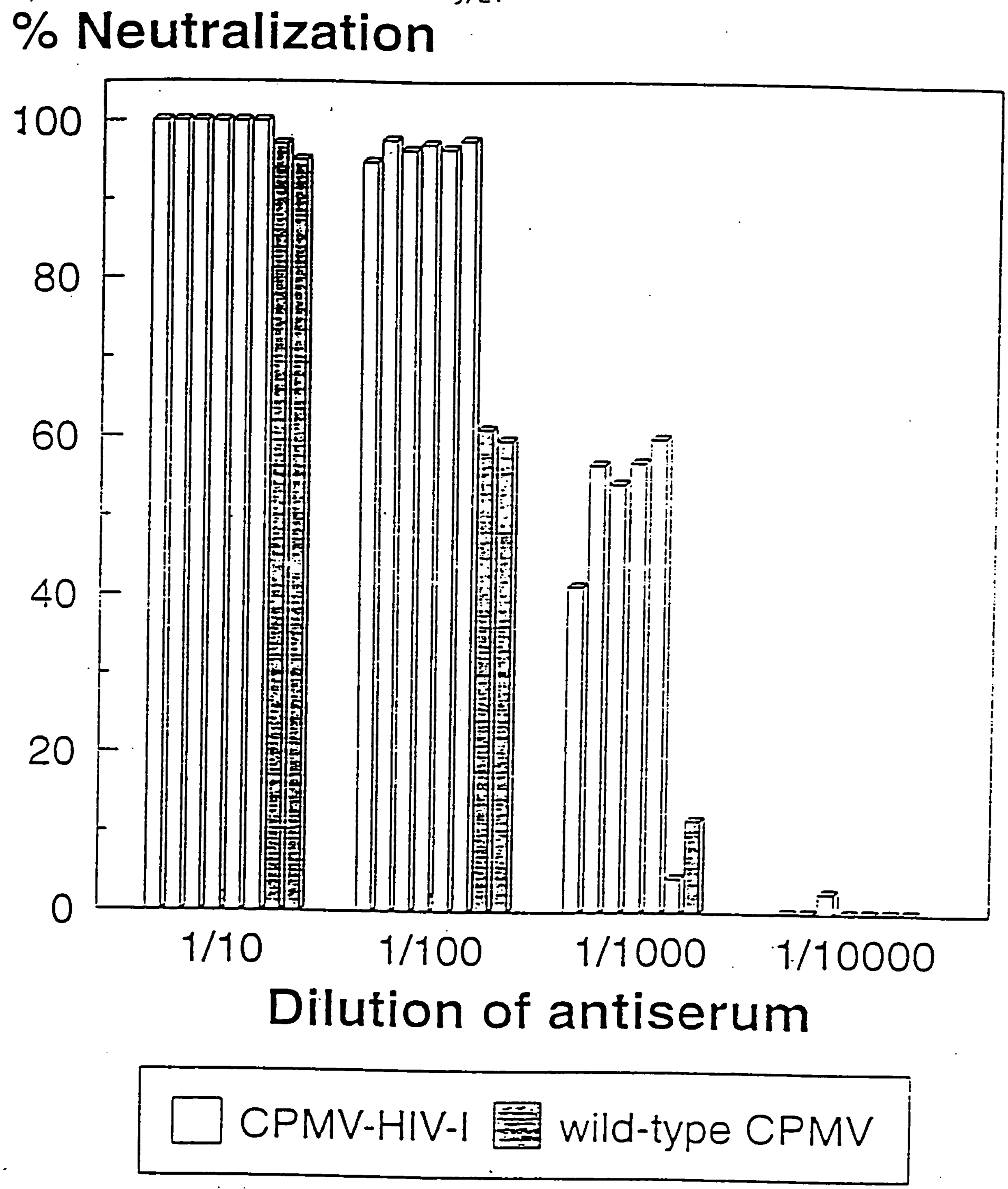
% Neutralization
100
80
60
40
20
0
1/10
1/100
1/1000
1/10000
Dilution of antiserum
CPMV-HIV-I
wild-type CPMV

FIGURE 9

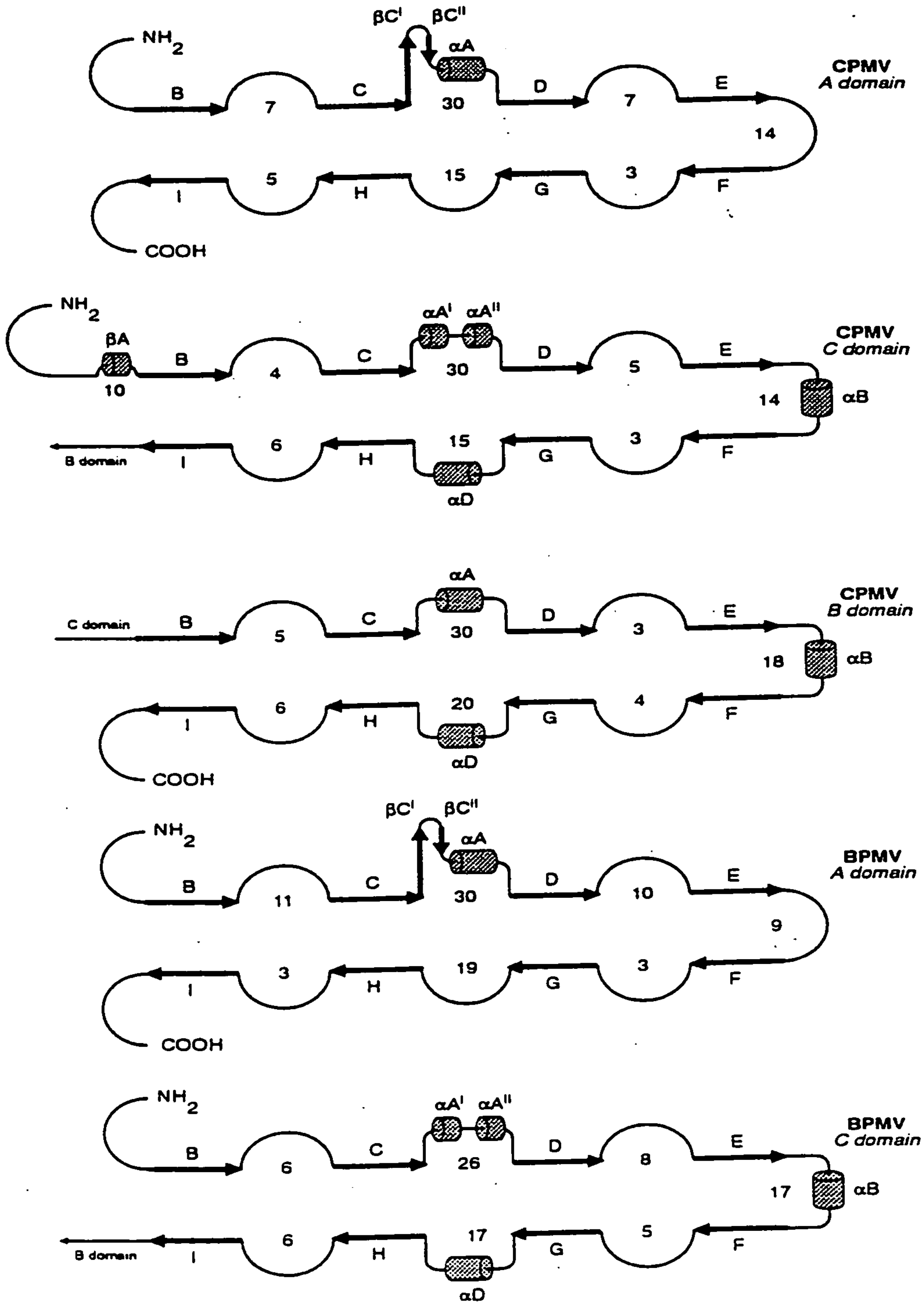
βCˡ βCˡˡ
NH2
αA
CPMV
A domain
B C D E
7 30 7 14
5 15 3
I H G F
COOH
NH2
αAˡ αAˡˡ
βA
CPMV
C domain
10 B 4 C 30 D 5 E
14 αB
B domain I 6 H 15 G 3 F
αD
αA
CPMV
B domain
C domain B 5 C 30 D 3 E
18 αB
I 6 H 20 G 4 F
αD
COOH
βCˡ βCˡˡ
NH2
αA
BPMV
A domain
B 11 C 30 D 10 E
9
I 3 H 19 G 3 F
COOH
NH2
αAˡ αAˡˡ
BPMV
C domain
B 6 C 26 D 8 E
17 αB
B domain I 6 H 17 G 5 F
αD

Figure 10

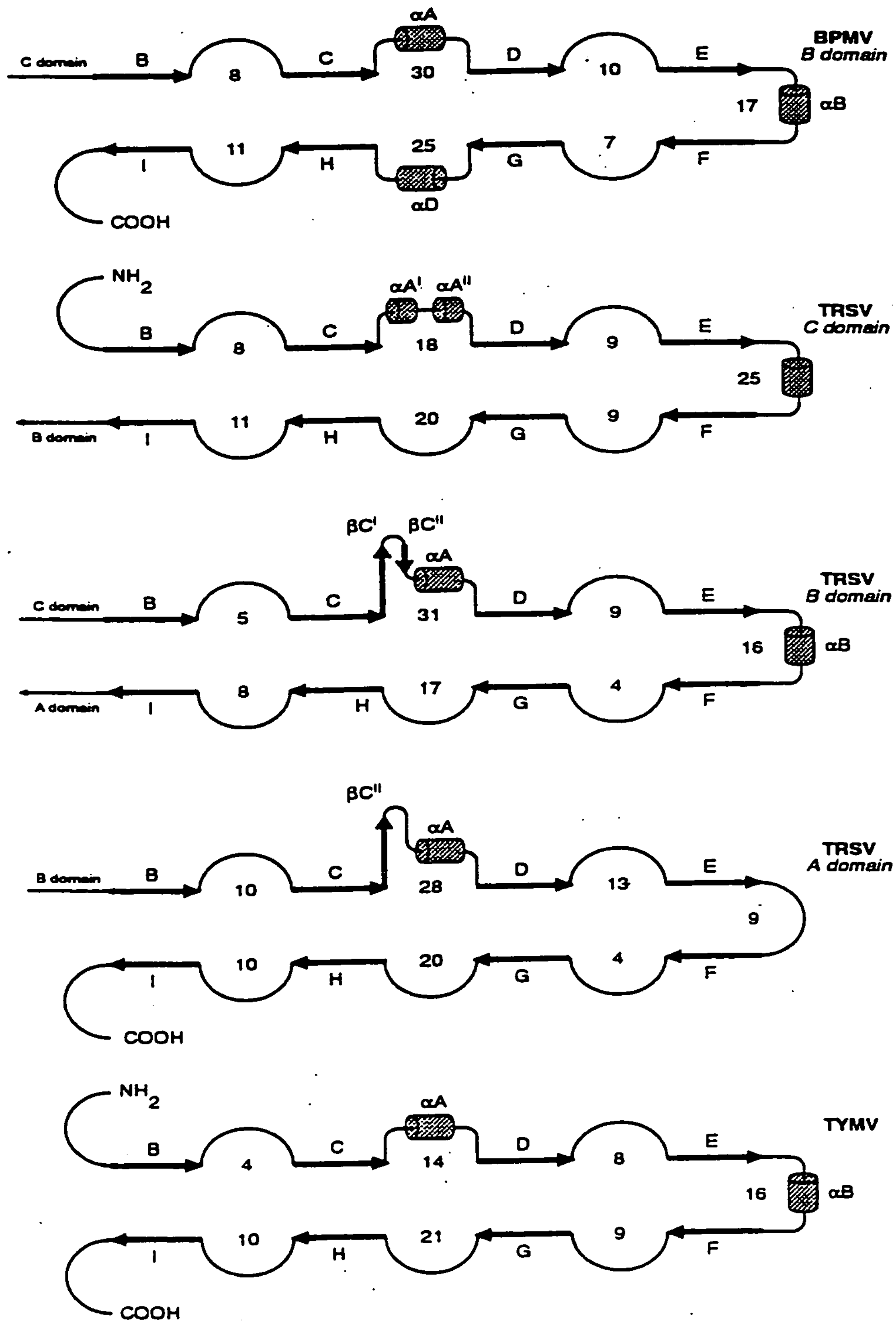
BPMV
B domain
C domain
COOH
TRSV
C domain
NH2
B domain
TRSV
B domain
C domain
A domain
TRSV
A domain
B domain
COOH
TYMV
NH2
COOH

Figure 10

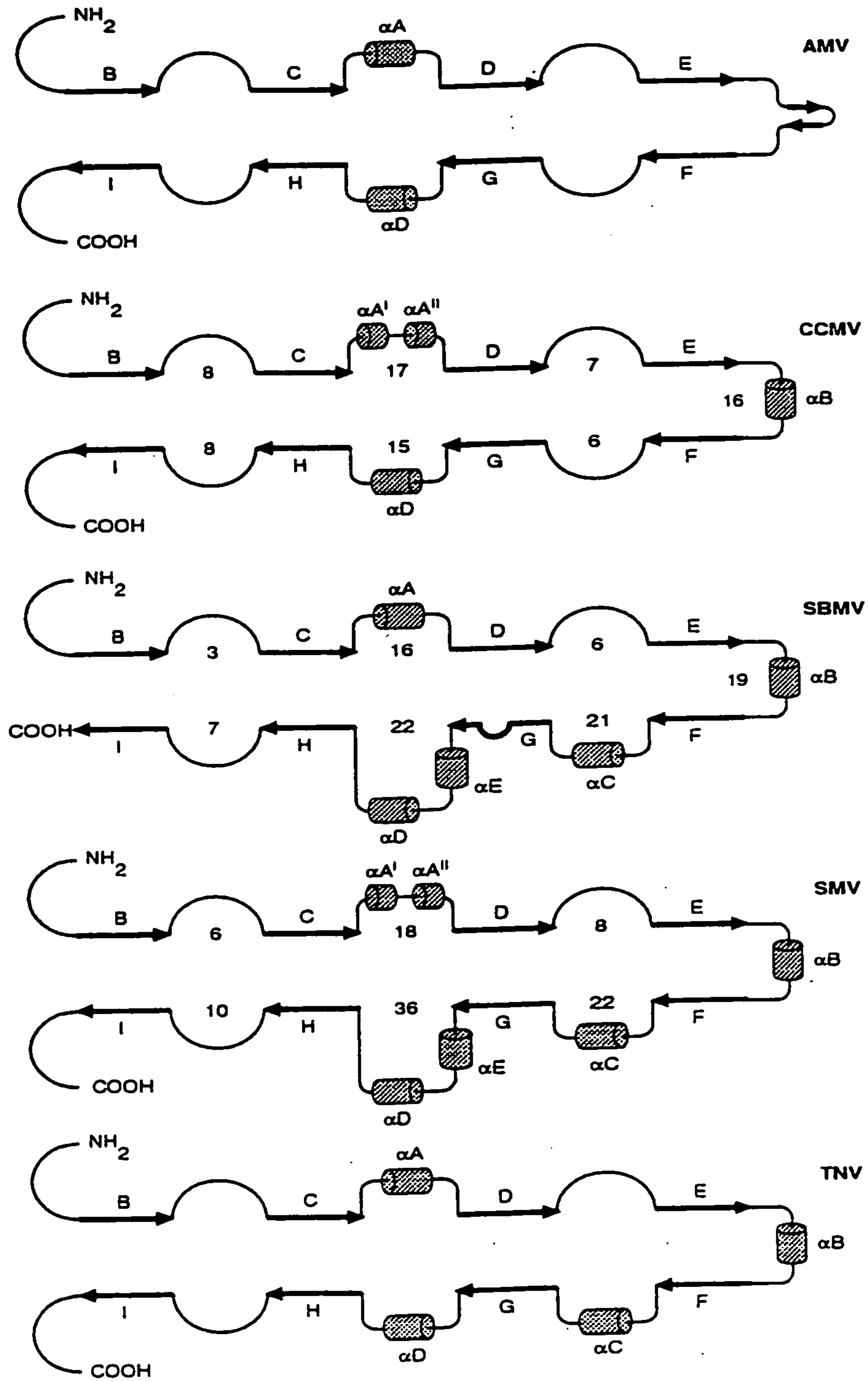

Figure 10

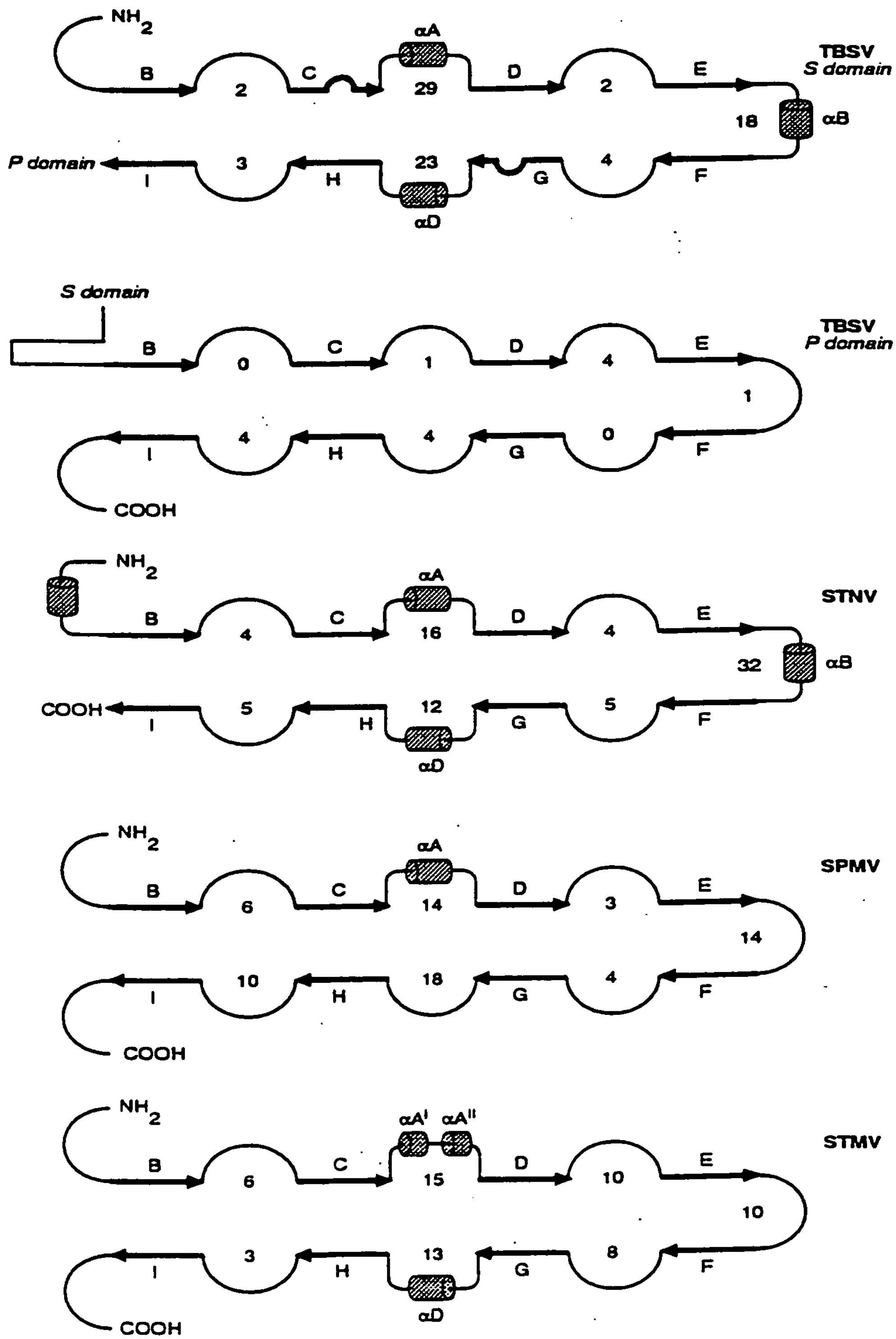
NH2
αA
TBSV
S domain
B
2
C
29
D
2
E
18
αB
P domain
I
3
H
23
G
4
F
αD
S domain
B
0
C
1
D
4
E
TBSV
P domain
1
I
4
H
4
G
0
F
COOH
NH2
αA
B
4
C
16
D
4
E
STNV
32
αB
COOH
I
5
H
12
G
5
F
αD
NH2
αA
B
6
C
14
D
3
E
SPMV
14
I
10
H
18
G
4
F
COOH
NH2
αA^I
αA^II
B
6
C
15
D
10
E
STMV
10
I
3
H
13
G
8
F
αD
COOH

Figure 10

Figure 11

(a) Sequence of SBMV Coat Protein Spanning The Potential Insertion Site With Introduced Base Changes and New Restriction Sites: (sequence starts at nt 3955)

M E G G S S K T A V N T G

ATGGAAGGAGGATC**A**TCTAAGACTGCTGT**G**AACACTGGG

GGATC**C** — *Bam*H I

GT**T**AAC — *Hpa* I

(b) Series of Sequences to be Inserted Between the Restriction Sites to Insert the MUC1(16) Epitope at Various Locations

G V T S A P D T R P A P G S T A

GGTGTTACTTCTGCTCCTGATACTAGACCTGCTCCTGGTTCTACTGCT
CCACAATGAAGACGACCACTATGATCTGGACGAGGACCAAGATGACGA

GATCC G	TCTAAGACTGCTGTT AGATTCTGACGACAA
GATCCTCT GAGA	AAGACTGCTGTT TTCTGACGACAA
GATCCTCTAAG GAGATTC	ACTGCTGTT TGACGACAA
GATCCTCTAAGACT GAGATTCTGA	GCTGTT CGACAA
GATCCTCTAAGACTGCT GAGATTCTGACGA	GTT CAA

Figure 12

```
LTSV  : NI---YAPARLTIAA-ANSSINIASVGTLYATYEVEL
SBMV  : NIGNILVPARLVIAMEGGSSKTAVNTGRLYASYTIRL
SMV   : NIATDLVPARLVIALLDGSSSTAVAAGRIYASYTIQM
                   #######=======##############
                     βH      loop       βI
```

Figure 13

(a) Sequence of LTSV Coat Protein Spanning The Potential Insertion Site With Introduced Base Changes and New Restriction Sites: (sequence starts at nt 3954)

I A A A N S S I N I A S V G T L Y

ATAGC**C**GCAGCTAACAGCTCCATAAACATAGCTAGTGTGGGTAC**T**CTTTAT

C**T**GCAG *Pst* I

GGTAC**C** *Kpn* I

(b) Series of Sequences to be Inserted Between the Restriction Sites to Insert the MUC1(16) Epitope at Various Locations.

G V T S A P D T R P A P G S T A

GGTGTTACTTCTGCTCCTGATACTAGACCTGCTCCTGGTTCTACTGCT
CCACAATGAAGACGACCACTATGATCTGGACGAGGACCAAGATGACGA

GCTAACAGC ACGTCGATTGTCG	TCCATAAACATAGCTAGTGTGGGTAC AGGTATTTGTATCGATCACACC
GCTAACAGCTCC ACGTCGATTGTCGAGG	ATAAACATAGCTAGTGTGGGTAC TATTTGTATCGATCACACC
GCTAACAGCTCCATA ACGTCGATTGTCGAGGTAT	AACATAGCTAGTGTGGGTAC TTGTATCGATCACACC
GCTAACAGCTCCATAAAC ACGTCGATTGTCGAGGTATTTG	ATAGCTAGTGTGGGTAC TATCGATCACACC
GCTAACAGCTCCATAAACATA ACGTCGATTGTCGAGGTATTTGTAT	GCTAGTGTGGGTAC CGATCACACC
GCTAACAGCTCCATAAACATAGCT ACGTCGATTGTCGAGGTATTTGTATCGA	AGTGTGGGTAC TCACACC

Figure 14

Lipman-Pearson alignment of RCNMV and TBSV coat protein sequences.

Lipman-Pearson Protein Alignment
Ktuple: 2; Gap Penalty: 4; Gap Length Penalty: 12

Seq1(1>389) tbsvtbs.PRO	Seq2(1>340) rcnmvdia.PRO	Similarity Index	Gap Number	Gap Length	Consensus Length
(64>387)	(8>338)	26.9	4	7	331

```
      70        80        90        100       110       120
KKQQMINHVGGTGGAIMAPVAVTRQLVGSKPKFTGRTSGSVTVTHREYLSQVNNSTGFQV
K.:Q. :. . T .: : .VA:. . .    . . ...  :.: H : :  V .S. .:.
KSKQRSQPRNRTPNTSVKTVAIPFAKTQIIKTVNPPPKPARGILHTQLVMSVVGSVQMRT
  10        20        30        40        50        60
      130       140       150       160       170       180
NGGIVGNLLQLNPLNGTLFSWLPAIASNFDQYTFNSVVLHYVPLCSTTEVGRVAIYFDKD
N.G  .: ::LNP N :LF: L:  A:N:D Y ::.:.L:YVPL :. : GRVA: .D D
NNGKSNQRFRLNPSNPALFPTLAYEAANYDMYRLKKLTLRYYVPLVTVQNSGRVAMIWDPD
  70        80        90        100       110       120
      190       200       210       220       230       240
SEDPEPADRVELANYSVLKETAPWAEAMLRVPTDKIKRFCDDSSTSDHKLIDLGQLGIAT
S:D:.P..R E::.YS   .TA ...  L :P:D:  RF .D::T D:KL:D:GQL :.T
SQDSAPQSRQEISAYSRSVSTAVYEKCSLTIPADNQWRFVADNTTVDRKLVDFGQLLFVT
  130       140       150       160       170       180
      250       260       270       280       290          300
YGGAGTNAVGDIFISYSVTLYFPQPTNTLLSTRRLDLAGALVTASGPGYLLVSR---TAT
.:G::. ..GDIF:...V.:   PQPT.::: . :DL:G:L.: .GP:YL: :    T::
HSGSDGIETGDIFLDCEVEFKGPQPTASIVQKTVIDLGGTLTSFEGPSYLMPPDAFITSS
  190       200       210       220       230       240
         310       320       330         340        350
VLTMTFRATGTFVISGTYRCLTATTLGLAG--GVNVNSITVVDNIG-TDSAFFINCTVSN
 :.:   .:GT:::: .  C T:.::.::G  .:  :: :. ::  ...S  F.:..V :
SFGLFVDVAGTYLLTLVVTCSTTGSVTVGGNSTLVGDGRAAYGSSNYIASIVFTSSGVLS
  250       260       270       280       290       300
  360        370       380
LPSVVTFT-STGITSATVHCVRATRQNDVSL
 .: V F: S:G::..  ::  R .: N.  L
TTPSVQFSGSSGVSRVQMNICRCKQGNTFIL
  310       320       330
```

Figure 15

Beta plot - Chou-Fasman

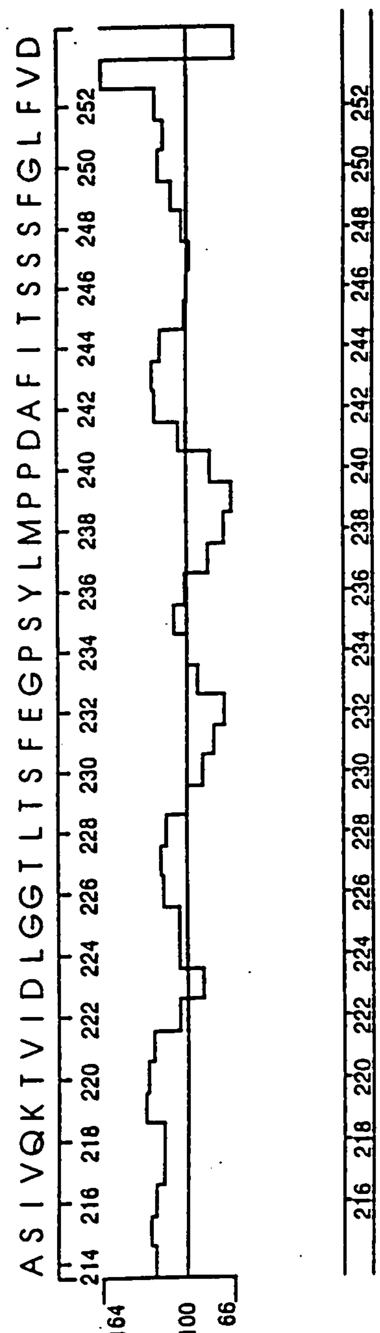

Figure 16

```
                           220        230        240
         AA       |ASIVQKYVIDLGGTLTSFEGPSYLMPP
         PHD sec  | HHHHHEEEE   EEEE    EEEEE
         Rel sec  |145432244525515625586487624
detail :
         prH sec  |466655321111100000000000000
         prE sec  |101123456632246752212688753
         prL sec  |422221112246642237787311246
subset : SUB sec  |..H......E.LL.EE.LLLL.EEE..
```

Abbreviations :

- AA : amino acid sequence
- H : helix
- E : extended (sheet)
- blank : other (loop)
- PHD : Profile network prediction HeiDelberg
- Rel : Reliability index of prediction (0-9)
- prH : probability for assigning helix
- prE : probability for assigning strand
- prL : probability for assigning loop
- SUB : a subset of the prediction, for all residues with an average expected accuracy of >82%

Figure 17

(a) Sequence of RCNMV Coat Protein Spanning The Potential Insertion Site With Introduced Base Changes and New Restriction Sites: (sequence starts at nt 3070)

S I V Q K T V I D L G G T L T S F
AGCATCGT**A**CA**G**AAAACTGTAATTGATCTCGGTGGGAC**AC**T**C**ACTTCTTTC

GT**G**CA**C** *ApaL*I

GTT**A**AC *Hpa*I

(b) Series of Sequences to be Inserted Between the Restriction Sites to Insert the MUC1(16) Epitope at Various Locations

G V T S A P D T R P A P G S T A
GGTGTTACTTCTGCTCCTGATACTAGACCTGCTCCTGGTTCTACTGCT
CCACAATGAAGACGACCACTATGATCTGGACGAGGACCAAGATGACGA

GAAAACTGTA ACGTCTTTTGACAT	ATTGATCTCGGTGGGACGTT TAACTAGAGCCACCCTGCAA
GAAAACTGTAATT ACGTCTTTTGACATTAA	GATCTCGGTGGGACGTT CTAGAGCCACCCTGCAA
GAAAACTGTAATTGAT ACGTCTTTTGACATTAACTA	CTCGGTGGGACGTT GAGCCACCCTGCAA
GAAAACTGTAATTGATCTC ACGTCTTTTGACATTAACTAGAG	GGTGGGACGTT CCACCCTGCAA
GAAAACTGTAATTGATCTCGGT ACGTCTTTTGACATTAACTAGAGCCA	GGGACGTT CCCTGCAA
GAAAACTGTAATTGATCTCGGTGGG ACGTCTTTTGACATTAACTAGAGCCACCC	ACGTT TGCAA

Figure 18

(a) Nucleotide and amino-acid sequence of the C-terminal region of the coat protein of TRV :

```
   S  T  P  A  S  G  G  S  G  A  T  P  P  P  A  S  G  G  A  V  R  P  N  P  *
1125 CGTCGACTCCGGCCTCGGGGGGAAGTGGTGCAACACCACCTCCTGCGAGTGGGGGTGCTGTGCGTCCTAATCCTTGATGTCGTCAAATCAAACCTTTAAGGGACCTT 1230
   ↑                                                                                                              ↑
   Sal I                                                                                                          PpuM I
```

(b) Series of sequences to be inserted between the *Sal* I and *PpuM* I restrictionsites to create C-terminal deletions :

```
S  T  P  A  S  G  G  S  G  A  T  P  P  P  A  S  G  G  A  *
TCGACTCCGGCCTCGGGGGGAAGTGGTGCAACACCACCTCCTGCGAGTGGGGGTGCTTGATGTCGTCAAATCAAACCTTTAAGG
    GAGGCCGGAGCCCCCCTTCACCACGTTGTGGTGGAGGACGCTCACCCCCACGAACTACAGCAGTTTAGTTTGGAAATTCCCTG

S  T  P  A  S  G  G  S  G  A  T  P  P  P  *
TCGACTCCGGCCTCGGGGGGAAGTGGTGCAACACCACCTCCTTGATGTCGTCAAATCAAACCTTTAAGG
    GAGGCCGGAGCCCCCCTTCACCACGTTGTGGTGGAGGAACTACAGCAGTTTAGTTTGGAAATTCCCTG

S  T  P  A  S  G  G  S  G  *
TCGACTCCGGCCTCGGGGGGAAGTGGTTGATGTCGTCAAATCAAACCTTTAAGG
    GAGGCCGGAGCCCCCCTTCACCAACTACAGCAGTTTAGTTTGGAAATTCCCTG

S  T  P  A  *
TCGACTCCGGCCTGATGTCGTCAAATCAAACCTTTAAGG
    GAGGCCGGACTACAGCAGTTTAGTTTGGAAATTCCCTG

S  T  *
TCGACTTGATGTCGTCAAATCAAACCTTTAAGG
    GAACTACAGCAGTTTAGTTTGGAAATTCCCTG
```